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(54) Title: STIMULATION OF TANNING BY DNA FRAGMENTS

(57) Abstract

A method of stimulating pigment production in mammalian skin, as well as protecting mammalian skin against ultraviolet damage, is disclosed. Also disclosed is a method of stimulating pigment production in mammalian cells, a method of stimulating melanogenesis in mammalian melanocytes, and a culture medium for stimulating melanin production. Preparations useful in the present methods are additionally disclosed. The methods comprise administering to the epidermis or to the cells DNA fragments, either single- or double-stranded, or a mixture of both, or deoxynucleotides, dinucleotides, or dinucleotide dimers, in an appropriate vehicle, such as a liposomal preparation or propylene glycol. The preparations include DNA fragments, deoxynucleotides, dinucleotides, or dinucleotide dimers and an appropriate delivery vehicle, such as liposomes or a propylene glycol. The DNA fragments, deoxynucleotides, or dinucleotides used in the methods or in the preparations can be ultraviolet-irradiated. The preparations are sunlight-independent tanning or melanogenesis-stimulating agent.

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Stimulation of tanning by DNA fragments.

Description

Background of the Invention

Human skin consists of two layers, the uppermost of which is the epidermis. The epidermis encompasses many different cell types, including melanocytes and keratinocytes. Melanocytes are specialized cells in the basal layer of the epidermis which synthesize melanin; the melanin is then packaged into melanosomes and then transported into keratinocytes.

It has been known for centuries that exposure of skin to the sun results in tanning, the skin's major form of endogenous protection against subsequent skin damage from ultraviolet (UV) irradiation. Melanin, a polymer which serves as a filter with absorbance within the UV range, provides photoprotection for the individual. The peak action spectrum for this phenomenon is in the UV-B range, 290-305 nm; various morphologic and enzymatic changes occur at the cellular level in epidermal melanocytes in response to UV irradiation. Proteins and nucleic acids of the epidermis absorb UV-B rays, causing the production of thymine dimers, which are known to be formed by UV irradiation of nuclear DNA and to be excised from the DNA strand by the action of highly specific enzymes, including endonucleases. If not removed, these dimers can stall DNA replication forks generating regions of single-stranded DNA. Failure to remove thymine dimers and other DNA mutations in the genome may lead to somatic mutations resulting in carcinogenesis.

In bacteria it is known that the DNA fragments released from stalled replication forks can interact with nuclear proteins which then regulate the expression of specific genes in the DNA as part of the organism's SOS response to UV damage. Bacteria do not tan, but tanning

might reasonably be considered part of the analogous SOS response in mammalian skin. The precise stimulus for UV-induced tanning, however, remains unknown.

Summary of the Invention

The current invention pertains to a method of increasing the pigmentation in skin. It consists of applying topically to the epidermis DNA fragments, either single- or double-stranded, or a mixture of both single- and double-stranded DNA fragments, or deoxynucleotides, dinucleotides, or dinucleotide dimers, notably in a delivery vehicle such as in a liposome preparation, such that the DNA fragments, deoxynucleotides, dinucleotides, or dinucleotide dimers are available to the melanocytes. The DNA fragments, either single- or double-stranded, or the mixture of both single-and double-stranded DNA fragments, or deoxynucleotides, or dinucleotides can be ultraviolet-irradiated. The method results in the stimulation of a tanning response equivalent to that produced by sun exposure, but without necessity of actual sun exposure, and thereby avoids subjecting the skin to the carcinogenic action of UV irradiation. The invention additionally pertains to a method of stimulating pigment production in melanocytes by applying to the cells DNA fragments, either single- or double-stranded, or a mixture of both single- and double-stranded DNA fragments, or deoxynucleotides, dinucleotides, or dinucleotide dimers. The invention further includes a culture medium for stimulating pigment production in melanocytes, comprising increasing melanin, the culture medium comprising an effective amount of DNA fragments, either single- or double-stranded, or a mixture of both single- and double-stranded DNA fragments, or deoxynucleotides, dinucleotides, or dinucleotide dimers. The invention also includes compositions useful in stimulating pigment

production in skin, comprising DNA fragments, deoxynucleotides, dinucleotides or dinucleotide dimers notably in an appropriate delivery vehicle, such as liposomes.

Application of DNA fragments, deoxynucleotides, dinucleotides, or dinucleotide dimers by the method of the present invention results in a cosmetically pleasing tan that may also be protective against subsequent UV damage to the skin, including sunburn, photoaging, and development of skin cancer. The results are obtained even in the absence of exposure to sunlight. The current invention can also be used for stimulating pigment production for other purposes, such as to increase pigmentation in hypopigmented areas of the skin.

Brief Description of the Figures

Figure 1 is a graph depicting the effect on pigmentation of the exposure of S91 cells to DNA fragments in a phospholipid carrier. CTRL = control (no liposomes or DNA fragments); A1 = empty liposomes (no DNA fragments); B1 = liposomes containing UV-DNA (irradiated); C1 = liposomes containing DNA (not irradiated).

Figure 2 is a graph depicting the effect on pigmentation of the exposure of S91 cells to DNA fragments. CTRL = control (no liposomes or DNA fragments); DNA = 100 μl (10 mg/ml) DNA; DNA/UV = 100 μl (10 mg/ml) DNA irradiated for 10 minutes with UVC; A1 = empty liposomes (no DNA fragments); B1 = liposomes containing UV-DNA (irradiated); C1 = liposomes containing DNA (not irradiated).

Figure 3 is a graph depicting the effect on pigmentation of the exposure of S91 cells to the dinucleotides $d(pT)_2$ and $d(pA)_2$ when cultured. CTRL = Control (no additions); IBMX = isobutylmethylxanthine (a

positive control); $d(pA)_2$ = deoxyadenylic acid dinucleotide; and $d(pT)_2$ = thymidylic acid dinucleotide.

Figure 4 is a graph depicting the effect on pigmentation of exposure of S91 cells to deoxydinucleotides $d(pA)_2$ and $d(pT)_2$ when cultured in a medium of DME plus 2% calf serum (CS). CTRL = Control (no additions); IBMX = isobutylmethylxanthine (a positive control); $d(pA)_2$ = deoxyadenylic acid dinucleotide; and $d(pT)_2$ = thymidylic acid dinucleotide.

Figure 5 is a graph depicting the effect on pigmentation of exposure of S91 cells to deoxynucleotides $d(pA)_2$ and $d(pT)_2$ and/or to IBMX when cultured in a medium of DME plus 2% calf serum (CS). CTRL = Control (water only); IBMX = isobutylmethylxanthine; A2 = $d(pA)_2$ (deoxyadenylic acid dinucleotide); T2 = $d(pT)_2$ (thymidylic acid dinucleotide); A2/IBMX = $d(pA)_2$ and IBMX; T2/IBMX = $d(pT)_2$ and IBMX.

Figure 6 a graph depicting the effect on pigmentation of exposure of S91 cells to deoxynucleotides $d(pA)_2$ and $d(pT)_2$ when cultured in a medium of DME plus 2% calf serum (CS). Control (water only); A2 = $d(pA)_2$ (deoxyadenylic acid dinucleotide); T2 = $d(pT)_2$ (thymidylic acid dinucleotide).

Figure 7 is a graph depicting the effect on pigmentation of exposure of S91 cells to deoxynucleotide pTdpC when cultured in a medium of DME plus 2% calf serum (CS). Control (water only); TC = d(pTpC).

Figure 8 is a graph depicting the effect on pigmentation of exposure of melanocytes cells to deoxynucleotides $d(pT)_2$ or to IBMX when cultured in a medium of DME plus 10% calf serum (CS). CTRL = Control; IBMX = isobutylmethylxanthine; T2 = $d(pT)_2$ (thymidylic acid dinucleotide).

Detailed Description of the Invention

The invention pertains to use of a preparation comprising DNA fragments, deoxynucleotides, dinucleotides, or dinucleotide dimers, as defined in the following description, for increasing pigmentation in mammalian skin by stimulating melanogenesis in melanocytes to form melanin pigments which are transferred to the keratinocytes. The invention also provides compositions comprising said DNA fragments, deoxynucleotides, dinucleotides, or dinucleotide dimers, for increasing skin pigmentation, as herein defined. The invention further comprises a method for stimulating skin pigment production comprising applying said DNA fragments, deoxynucleotides, dinucleotides, or dinucleotide dimers, as herein defined. No exposure to sunlight is necessary for the increase in pigmentation resulting from the uses, methods, and compositions of the current invention. The invention is thus useful for sunlight-independent skin tanning, as well as for pigmenting skin grafts, allografts, and autografts, both *in vivo* and *in vitro*, and also for treating hypopigmentation disorders such as vitiligo, albinism, piebaldism, and post-inflammatory hypopigmentation. Other advantageous invention embodiments are set forth in the claims, the content thereof being incorporated in its entirety in the present description by reference.

In one embodiment, DNA fragments, of approximately 2-200 bases in length, deoxynucleotides (single bases), dinucleotides, or dinucleotide dimers, are administered topically to the epidermis, in an appropriate vehicle, such as a liposome preparation or propylene glycol, in a quantity sufficient to stimulate melanin production. As used herein, "DNA fragments" refers to single-stranded DNA fragments, double-stranded DNA fragments, a mixture of both single- and double-stranded DNA fragments, or deoxynucleotides, dinucleotides, or dinucleotide dimers.

"Deoxynucleotides" refers to either a single type of deoxynucleotide or a mixture of different deoxynucleotides. "Dinucleotides" can comprise a single type of nucleotide or different types of nucleotides, and can comprise different types of dinucleotides; in a preferred embodiment, the nucleotides of the dinucleotides are deoxynucleotides. Representative dinucleotides include $d(pT)_2$, $d(pC)_2$, $d(pA)_2$, d(pCpT), d(pTpC), d(CpT), d(TpC) and d(TpT), where T is thymine, C is cytosine, A is adenosine, d is deoxy, and p is phosphate (see Niggli, *Photochem. Photobiol. 38(3)*:353-356 (1988)). A combination of at least two or more of DNA fragments, deoxynucleotides, dinucleotides, and/or dinucleotide dimers can also be used. The DNA fragments, deoxynucleotides, or dinucleotides can be ultraviolet-irradiated. Such ultraviolet irradiation usually results in photodimerization between two adjacent pyrimidine residues (i.e., thymine (T) and cytosine (C)) present in the DNA fragments, or dinucleotides. One skilled in the art may refer to Biochemistry, (J.D. Rawn, Nail Patterson, Publisher, Carolina Biological Supply Company, North Carolina, 1989, pages 730-736). The DNA fragments, deoxynucleotides, dinucleotides, and dinucleotide dimers utilized in the current invention act as sunlight-independent active agents: the stimulation of pigmentation results even in the absence of exposure to sunlight.

The DNA fragments, deoxynucleotides, dinucleotides, or dinucleotide dimers can be obtained from any appropriate source, or can be synthetic DNA fragments, deoxynucleotides, dinucleotides, or dinucleotide dimers. For example, salmon sperm DNA can be dissolved in water, and then the mixture can be autoclaved to fragment the DNA. The delivery vehicle can be any appropriate vehicle

which promotes the delivery of the DNA fragments, deoxynucleotides, dinucleotides, or dinucleotide dimers to the melanocytes. In one embodiment, a liposome preparation is used. The liposome preparation can be comprised of any liposomes which penetrate the stratum corneum and fuse with the cell membrane, resulting in delivery of the contents of the liposome into the cell. For example, liposomes such as those described in U.S. Patent No. 5,077,211 of Yarosh, U.S. Patent No. 4,621,023 of Redziniak et al. or U.S. Patent No. 4,508,703 of Redziniak et al. can be used.

Alternatively, the DNA fragments, deoxynucleotides, dinucleotides or dinucleotide dimers can be applied directly to the epidermis. In addition, the DNA fragments, deoxynucleotides, dinucleotides, or dinucleotide dimers can be applied in a vehicle which specifically targets melanocytes. For example, a membrane marker specific for melanocytes, such as melanocyte stimulating hormone (MSH), can be incorporated into a liposome containing the DNA fragments, deoxynucleotides, dinucleotides or dinucleotide dimers. Furthermore, according to an advantageous invention embodiment, the DNA fragments, deoxynucleotides, dinucleotides or dinucleotide dimers are used in combination with a diacyl glycerol. Preferred diacyl glycerols are 1,2-dioctanoyl glycerol, 1,2-didecanoyl glycerol, and 1-oleoyl-2-acetyl-glycerol. A preferred concentration of diacyl glycerol will usually range from 0.10 to 20.0 mM. In another advantageous embodiment, the DNA fragments, deoxynucleotides, dinucleotides or dinucleotide dimers are used in combination with an agent that stimulates the cyclic AMP pathway, such as isobutylmethylxanthine (IBMX). A preferred concentration of IBMX will usually range from 0.10 to 20.0 mM. Alternatively, the IBMX can be applied

subsequently to the application of the DNA fragments, deoxynucleotides, dinucleotides or dinucleotide dimers.

The delivery vehicle can also contain perfumes, colorants, stabilizers, sunscreens, or other ingredients. The preparation is applied topically to the skin surface at regular intervals, such as once or twice daily, in a suitable vehicle at an effective concentration, which will generally be approximately 0.1 μM to 1 mM or 0.05-10 mg/ml. Representative preferred concentrations include 100-300 μM, or 25-500 μM. The concentration will depend on the molecular weight of the fragments employed.

A similar such method is used to stimulate pigment production in mammalian cells, and particularly in melanocytes. In one embodiment, DNA fragments, either single- or double-stranded, or a mixture of both single- and double-stranded DNA fragments, or deoxynucleotides, dinucleotides, or dinucleotide dimers are applied to melanocytes in an appropriate vehicle, such as a liposome preparation or propylene glycol. It is believed that the increase in pigmentation results from stimulating melanogenesis by penetration of the invention DNA fragments inside the melanocytes and even inside the nuclei of the cells.

The current invention further encompasses a culture medium for stimulating melanin production in mammalian melanocytes, the medium comprising an effective amount of DNA fragments, either single-stranded, double-stranded, or a mixture of single- and double-stranded DNA fragments, deoxynucleotides, dinucleotides, or dinucleotide dimers. The DNA fragments, deoxynucleotides, or dinucleotides can also be ultraviolet-irradiated. The effective amount will generally be between 0.1-1 μM.

The invention is further illustrated by the following Examples.

EXAMPLE 1 Effect of DNA Fragments on the Pigmentation of S91 Cells

DNA for all experiments was fragmented salmon sperm DNA (Sigma Chemical Co.). Liposome preparations were obtained from the company Parfums Christian Dior.

In one experiment, S91 cells (a murine melanoma cell line) were plated at 2 x 10^5 cells per dish on 60 mm diameter culture dishes, in DME plus 10% calf serum (CS) at 37°C, and cultured for 24 hours (Day 1). On Day 2, the medium was changed, and cells were exposed to one of three additions: 100 μM empty liposomes (A_1); 100 μM liposomes containing UV-DNA (irradiated) (B_1); and 100 μM liposomes containing DNA (not irradiated) (C_1). The cells were then cultured for three days; on Day 5, the medium was changed and the cells were cultured for another three days in the presence of the respective additions. On Day 8 the cells were collected, and assayed for melanin.

The results of this experiment, as shown in Figure 1, indicate that the addition of liposomes containing UV-DNA stimulated melanin production to a greater extent than the addition of empty liposomes or liposomes containing non-irradiated DNA; the addition of liposomes containing non-irradiated DNA stimulated melanin production in comparison to empty liposomes and the control.

In a second experiment, S91 cells were plated at 3 x 10^5 cells per dish on 60 mm culture dishes, in DME plus 10% CS at 37°C, and cultured for 24 hours (Day 1). On Day 2, the medium was changed, and cells were exposed to one of five additions: 100 μM empty liposomes (A_1); 100 μM liposomes containing UV-DNA (irradiated) (B_1); 100 μM liposomes containing DNA (not irradiated) (C_1); 100 μl (10 mg/ml) DNA (non-encapsulated (i.e. not within liposomes), and not irradiated); or 100 μl (10 mg/ml) DNA plus 10 minutes of UVC (non-encapsulated, and irradiated). The

cells were then cultured for two days; on Day 4, the medium was changed and the cells were cultured for another three days in the presence of the respective additions. On Day 7 the cells were collected, and assayed for melanin.

The results of this experiment, as shown in Figure 2, indicate that the addition of liposomes containing UV-DNA stimulated melanin production to a greater extent than the addition of empty liposomes, liposomes containing non-irradiated DNA, non-encapsulated DNA, or non-encapsulated UV-irradiated DNA. The addition of liposomes containing non-irradiated DNA stimulated melanin production to a greater extent than did the addition of empty liposomes, non-encapsulated DNA, or non-encapsulated UV-irradiated DNA.

In a third experiment, S91 cells were plated on 60 mm culture dishes in DME plus 10% calf serum (CS) at a density of 2 x 10^5 cells/dish. Two days later, the medium was changed and fresh DME plus 10% CS was added, along with either 50 μM deoxyadenylic acid dinucleotide ($d(pA)_2$), 50 μM thymidylic acid dinucleotide ($d(pT)_2$) or 100 μM isobutylmethylxanthine (IBMX), a known stimulator of melanogenesis in S91 cells, as a positive control. Four days later, the cells were collected and counted and an equal number of cells was pelleted for calculation of melanin/cell based on OD_{475}. Figure 3 shows that while 50 μM $d(pA)_2$ yielded a 50% increase in melanin/cell compared to nontreated controls, $d(pT)_2$ gave a 7-fold increase. As is normally observed, IBMX stimulated pigmentation approximately 15-fold above background.

In a fourth experiment, S91 cells were plated at a density of 20 x 10^5 cells/dish and grown for 3 days in DME plus 10% CS. On the fourth day, the medium was changed to DME plus 2% CS to slow cell proliferation. At this time,

plates were either non-supplemented (controls) or given 50 μM or 100 μM IBMX, $d(pA)_2$ or $d(pT)_2$. After 3 days, the cells were collected and the melanin/cell calculated. The results are represented in Figure 4. The cells exposed to $d(pT)_2$ showed a dose-dependent increase in melanin/cell with 50 μM $d(pT)_2$ and 100 mm $d(pT)_2$ showing a 13 and 30-fold increase respectively compared to controls. Cells exposed to $d(pA)_2$ showed a three to four-fold increase in melanin content. 100 μM IBMX gave a nearly 15-fold increase in pigmentation above the negative control, as expected.

In a fifth experiment, S91 cells were plated on 60 mm culture dishes in DME plus 2% calf serum (CS) at a density of 2 x 10^5 cells/dish. Plates were dosed with either 150 μl water (control), 100 μM $d(pA)_2$, or 100 μM $d(pT)_2$. Four days later, the cells were collected and the melanin/cell was calculated. Figure 5 shows that both $d(pA)_2$ and $d(pT)_2$ gave an increase in melanin over the control.

In a sixth experiment, S91 cells were plated at a density of 1 x 10^5 cells/dish and grown in DME plus 2% CS. On Day 1, a first (control) plate and a second plate were each given 150 μl water; a third and fourth plate were each given 150 μl $d(pT)_2$ stock solution (final concentration of dinucleotides was 100 μM); and a fifth and sixth plate were each given 150 μl $d(pA)_2$ stock solution (final concentration of dinucleotides was 100 μM). On Day 3, the second, fourth and sixth plates were dosed with 100 μM IBMX, and the first (control), third and fifth plates were non-supplemented. On Day 6, the cells were collected and the melanin/cell calculated. The results are represented in Figure 6. The cells exposed to IBMX alone, $d(pT)_2$, or $d(pA)_2$ showed an increase in melanin/cell compared to controls. Cells exposed to

$d(pA)_2$/IBMX or $d(pT)_2$/IBMX showed an increase in melanin content over the cells exposed to IBMX, $d(pT)_2$, or $d(pA)_2$ alone. Cells exposed to $d(pA)_2$/IBMX, and particularly to $d(pT)_2$/IBMX demonstrated a synergistic (greater than additive) increase in melanin.

In a seventh experiment, S91 cells were plated on 60 mm culture dishes in DME plus 2% calf serum (CS) at a density of 1 x 10^5 cells/dish. Plates were dosed with either 150 μl water (control) or 100 μM d(pTpC). Six days later, the cells were collected and the melanin/cell was calculated. Figure 7 shows that dTpdC gave a significant increase in melanin over the control.

In further testing carried out using a similar protocol, the S91 cells were dosed with TpT. Preliminary results indicated that TpT showed no significant increase of the melanin/cell over the control level.

EXAMPLE 2 Effect of DNA Fragments on the Pigmentation of Melanocytes

Human melanocytes obtained from a black donor were cultured in DME plus 10% CS, 20 ng/ml epidermal growth factor (EGF), 10 ng/ml fibroblast growth factor (FGF), and 1.2 nM cholera toxin. On day 1, plates were exposed to one of four treatments: nothing (control), 100 μM IBMX, 100 μM $d(pT)_2$, or 100 nm $d(pT)_2$. On days 2 and 3, plates were given fresh medium and another dose of the same treatment as on day 1. On day 7, the cells were collected and the melanin/cell calculated. The results are represented in Figure 8. The cells exposed to IBMX alone, or $d(pT)_2$ showed an increase in melanin/cell compared to the control.

EXAMPLE 3 Effect of DNA Fragments on the Pigmentation of Guinea Pig Epidermis

Two guinea pigs (designated Animal G and Animal H) were exposed to a series of solutions to determine the effect of thymidine dinucleotides on skin pigmentation. The number assigned to each solution, and the composition of the solution, are shown below in the Table.

Solutions were applied twice daily for five consecutive days. Each solution was applied to a separate region, identified as an "application site", of the dorsal epidermis of each animal. Animals were denuded prior to the first application and upon subsequent observations. The animals were maintained during the experiment in a confined room under normal, natural daylight or normal, artificial daylight, excluding any specific exposure to ultraviolet irradiation. Animals were photographed prior to the first application (day 1), during the experiment (days 7, 14, 21), and prior to surgical biopsies (day 26). The animals were observed for changes, and especially increases, in skin pigmentation; a scale ranging from 0 (no change in pigmentation) to +3 (greatest increase in pigmentation), having increments of 0, +/-, +1, +2, and +3, was used to grade the changes of skin pigmentation. The results of the experiment are shown below in the Table.

TABLE Solutions Utilized in Guinea Pig Study

Sol. No.	Composition of Solution	Animal	Changes in Skin Pigmentation			
			Day 7	Day 14	Day 21	Day 26
1	PG vehicle control	G	0	0	0	0
		H	0	0	+/-	+1
2	3:1 PG:DMSO vehicle control	G	0	0	+/-	+/-
		H	*	0	+/-	+1
3	100 μM d(pT)$_2$ in PG	G	0	0	+/-	+/-
		H	*	0	+/-	+2
4	100 μM d(pT)$_2$ in 3:1 PG:DMSO	G	0	0	+/-	+/-
		H	*	0	+/-	+2
5	300 μM d(pT)$_2$ in PG	G	0	0	+/-	+1
		H	*	0	+/-	+1
6	300 μM d(pT)$_2$ in 3:1 PG:DMSO	G	0	0	+/-	+1
		H	*	0	+1	+2

Sol. No. = solution number; PG = propylene glycol; DMSO = dimethyl sulfoxide.
* = slight irritation.

As shown in the Table, both animals demonstrated a slight increase in pigmentation on day 21, and a more pronounced increase in pigmentation on day 26. Biopsies from an untreated site, as well as from the sites for solutions 1 and 3 of Animal H, indicated greater concentration of melanin in the site treated with solution 3 (100 μM p(dT)$_2$ in propylene glycol) than in either the untreated site or the site treated with the control PG solutions (data not shown).

EXAMPLE 4 Emulsion of the Current Invention

Three different phases were combined to form the preparation.

Phase A:

Salmon sperm DNA fragments	0.2 g
Deoxynucleotide d(TpT)	0.02 g
Distilled water	10 g

Phase B:

1-oleoyl-2-acetyl-glycerol	0.5 g
Miglyol 812	10g

Phase C:

Excipient for emulsion
(including Miglyol 812 10% and Carbopol 1342 2%)
sufficient for 100 g

The water solution of salmon sperm DNA fragments was prepared by a standard autoclaving process. Phase A was prepared by mixing the components at room temperature. A moderate heating at 30°C was preferred for preparing Phase B. Phase A and Phase B were emulsified into Phase C by standard emulsifying processes. Autoclaving and emulsifying processes are well known to those skilled in the art. The resulting emulsion, when applied on the skin, will stimulate tanning even without specific sunlight exposure.

EXAMPLE 5 Liposomal Gel of the Current Invention

Two different phases were combined to form the preparation.

Phase A:

Soya phospholipids	0.9 g
Beta-sitosterol	0.1 g
Deoxynucleotide $d(pT)_2$	0.001 g
Water sufficient for	50 g

Phase B:

Carbopol 941 gel at 2% in water
Gel sufficient for 50 g

In a first step, a spray-dried powder is prepared from a solution of phospholipids and beta-sitosterol (phase A) according to the process described in US Patent No. 4,508,703. This powder is then dispersed into an aqueous solution of the other ingredients in Phase A, stirred during one hour, and homogenized under pressure, according to the process described in the US Patent No. 4,621,023 to obtain a liposomal suspension.

The liposomal suspension is then mixed with the same weight of Carbopol gel (Phase B).

The resulting liposomal gel can be applied, preferably once a day in the morning, on the skin to obtain a cosmetic tanning response or protection against the nocive effect of sun, irrespective of sun exposure.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described specifically herein. Such equivalents are intended to be encompassed in the scope of the following claims.

CLAIMS

What is claimed is:

1. Use of DNA fragments selected from the group consisting of: single-stranded DNA fragments, double-stranded DNA fragments, a mixture of single- and double-stranded DNA fragments, deoxynucleotides, dinucleotides,and dinucleotide dimers, for the manufacture of a medicament for protecting the skin of a mammal against ultraviolet damage, for instance sunburn, photoaging or skin cancer, or for stimulating pigmentation.

2. Use of DNA fragments selected from the group consisting of: single-stranded DNA fragments, double-stranded DNA fragments, a mixture of single- and double-stranded DNA fragments, deoxynucleotides, dinucleotides, and dinucleotide dimers, as cosmetic agents for stimulating pigmentation of the skin.

3. The use of Claim 1 or 2, wherein the DNA fragments are approximately 2-200 bases in length.

4. The use of Claim 1 or 2, wherein the dinucleotides are selected from the group consisting of: $d(pT)_2$, $d(pC)_2$, $d(pA)_2$, d(pCpT), d(pTpC), d(CpT) and d(TpC).

5. The use according to any one of claims 1 to 4, wherein said single-stranded DNA fragments, double-stranded DNA fragments, a mixture of single- and double-stranded DNA fragments, deoxynucleotides, or dinucleotides are ultraviolet-irradiated.

6. The use of Claim 1 or 2, wherein the dinucleotide dimers are dimers of dinucleotides selected from the group consisting of: $d(pT)_2$, $d(pC)_2$, $d(pA)_2$, d(pCpT), d(pTpC), d(CpT) and d(TpC).

7. The use according to any one of Claims 1 to 6, wherein said DNA fragments are present in a delivery vehicle promoting delivering of said DNA fragments to melanocytes.

8. The use of Claim 8, wherein said delivery vehicle comprises liposomes.

9. The use of Claim 8, wherein said delivery vehicle comprises propylene glycol.

10. Use according to any one of Claims 1 to 9, wherein the concentration of said DNA fragments ranges between 0.05 and 10 mg/ml.

11. Use according to any one of Claims 1 to 9, wherein the concentration of said DNA fragments ranges between 0.1 μM and 1 mM.

12. Use according to any one of Claims 1 to 9, wherein the concentration of said DNA fragments ranges between 25 μM and 500 μM.

13. Use according to any one of the preceding claims, wherein said mammal is a human.

14. Use according to any one of the preceding claims, wherein said DNA fragments are present in combination with a diacyl glycerol.

15. Use according to Claim 14, wherein the diacyl glycerol is selected from the group consisting of: 1,2-dioctanoyl glycerol, 1,2-didecanoyl glycerol, and 1-oleoyl-2-acetyl-glycerol.

16. Use according to any one of Claims 1 to 13, wherein said DNA fragments are present in combination with isobutylmethylxanthine.

17. Use according to any one of the preceding Claims, wherein said DNA fragments are sunlight-independent active agents.

18. A composition for protecting the skin of a mammal against ultraviolet damage, for example, sunburn, photoaging or skin cancer, or for stimulating pigmentation of skin, comprising DNA fragments selected from the group consisting of: single-stranded DNA fragments, double-stranded DNA fragments, a mixture of single- and double-stranded DNA fragments, deoxynucleotides, dinucleotides, and dinucleotide dimers.

19. The composition of claim 18, wherein said DNA fragments are approximately 2-200 bases in length.

20. The composition of Claim 18, wherein said dinucleotides are selected from the group consisting of: $d(pT)_2$, $d(pC)_2$, $d(pA)_2$, d(pCpT), d(pTpC), d(CpT) and d(TpC).

21. The composition of any one of Claims 18 to 20, wherein said single-stranded DNA fragments, double-stranded DNA fragments, a mixture of single- and double-

stranded DNA fragments, deoxynucleotides, or dinucleotides are ultraviolet-irradiated.

22. The composition of Claim 18, wherein said dinucleotide dimers are dimers of dinucleotides selected from the group consisting of: $d(pT)_2$, $d(pC)_2$, $d(pA)_2$, d(pCpT), d(pTpC), d(CpT) and d(TpC).

23. The composition according to any one of Claims 18 to 22, wherein said DNA fragments are present in a delivery vehicle promoting delivering of said DNA fragments to melanocytes.

24. The composition of Claim 23, wherein said delivery vehicle comprises liposomes.

25. The composition of Claim 23, wherein said delivery vehicle comprises propylene glycol.

26. The composition according to any one of Claims 18 to 25, wherein the concentration of said DNA fragments ranges between 0.1 μM and 1 mM.

27. The composition according to any one of Claims 18 to 26, wherein said composition further comprises a diacyl glycerol.

28. The composition according to Claim 27, wherein said diacyl glycerol is selected from the group consisting of: 1,2-dioctanoyl glycerol, 1,2-didecanoyl glycerol, and 1-oleoyl-2-acetyl-glycerol.

29. The composition according to any one of claims 17 to 26 wherein said DNA fragments are present in combination with isobutylmethylxanthine.

30. The composition according to any one of Claims 18 to 29, wherein said DNA fragments are sunlight-independent active agents.

31. A culture medium comprising an effective amount of DNA fragments selected from the group consisting of: single-stranded DNA fragments, double-stranded DNA fragments, a mixture of single- and double-stranded DNA fragments, deoxynucleotides, dinucleotides, and dinucleotide dimers, for stimulating melanin production in mammalian melanocytes when cultivating said melanocytes with said culture medium.

32. The culture medium of Claim 31, wherein said DNA fragments are present in a concentration ranging from 0.1 mM to 1 mM.

33. The culture medium of Claims 31 or 32, wherein said single-stranded DNA fragments, double-stranded DNA fragments, a mixture of single- and double-stranded DNA fragments, deoxynucleotides, or dinucleotides are ultraviolet-irradiated.

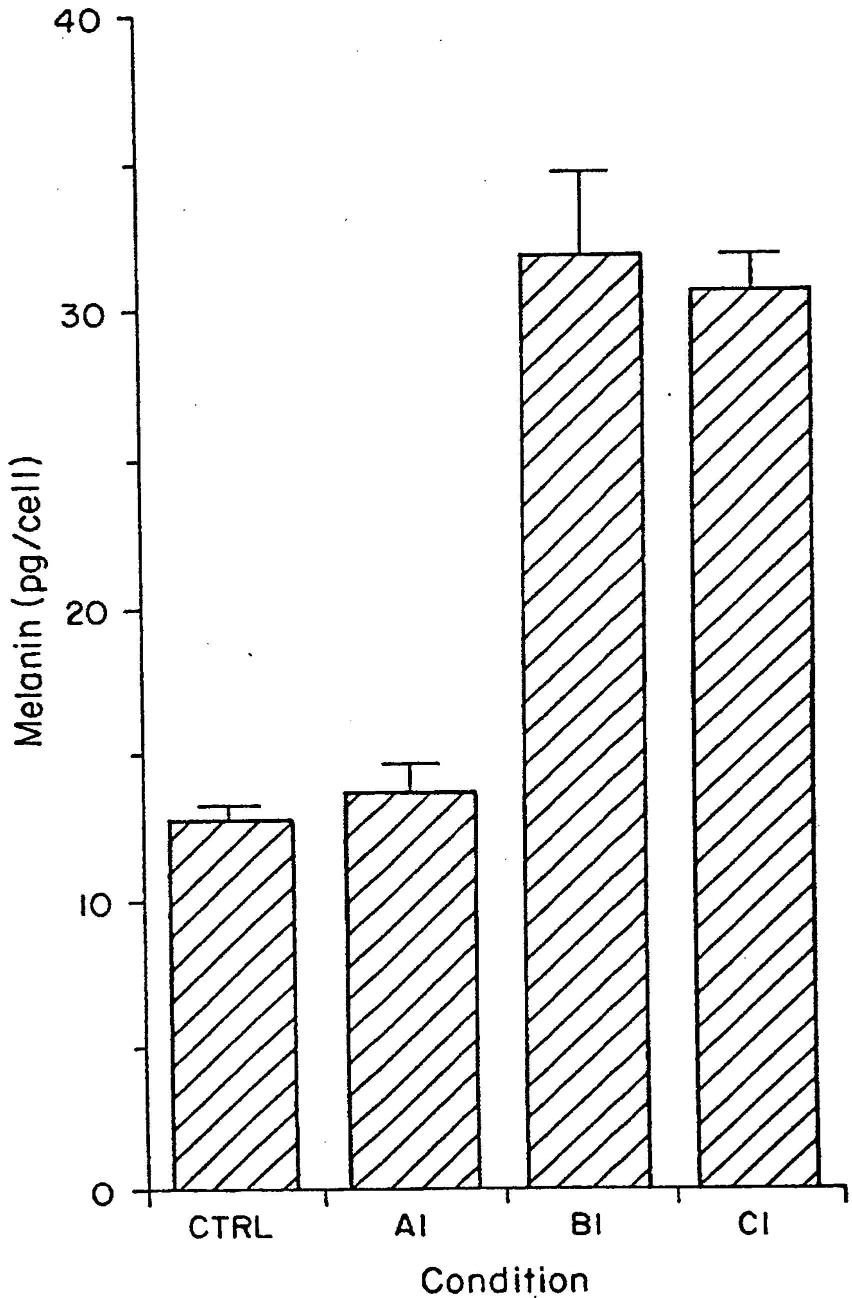

FIG. 1

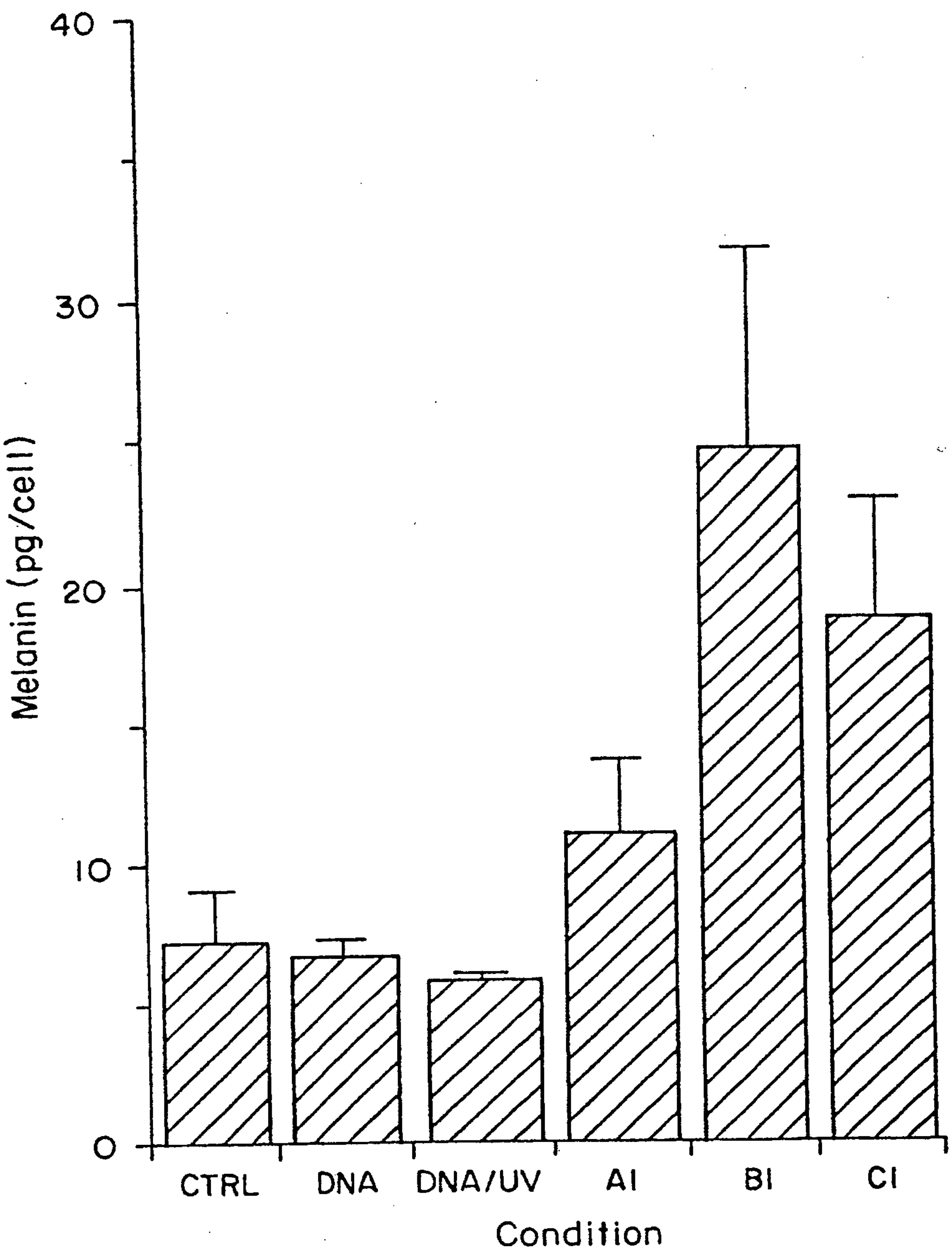

FIG. 2

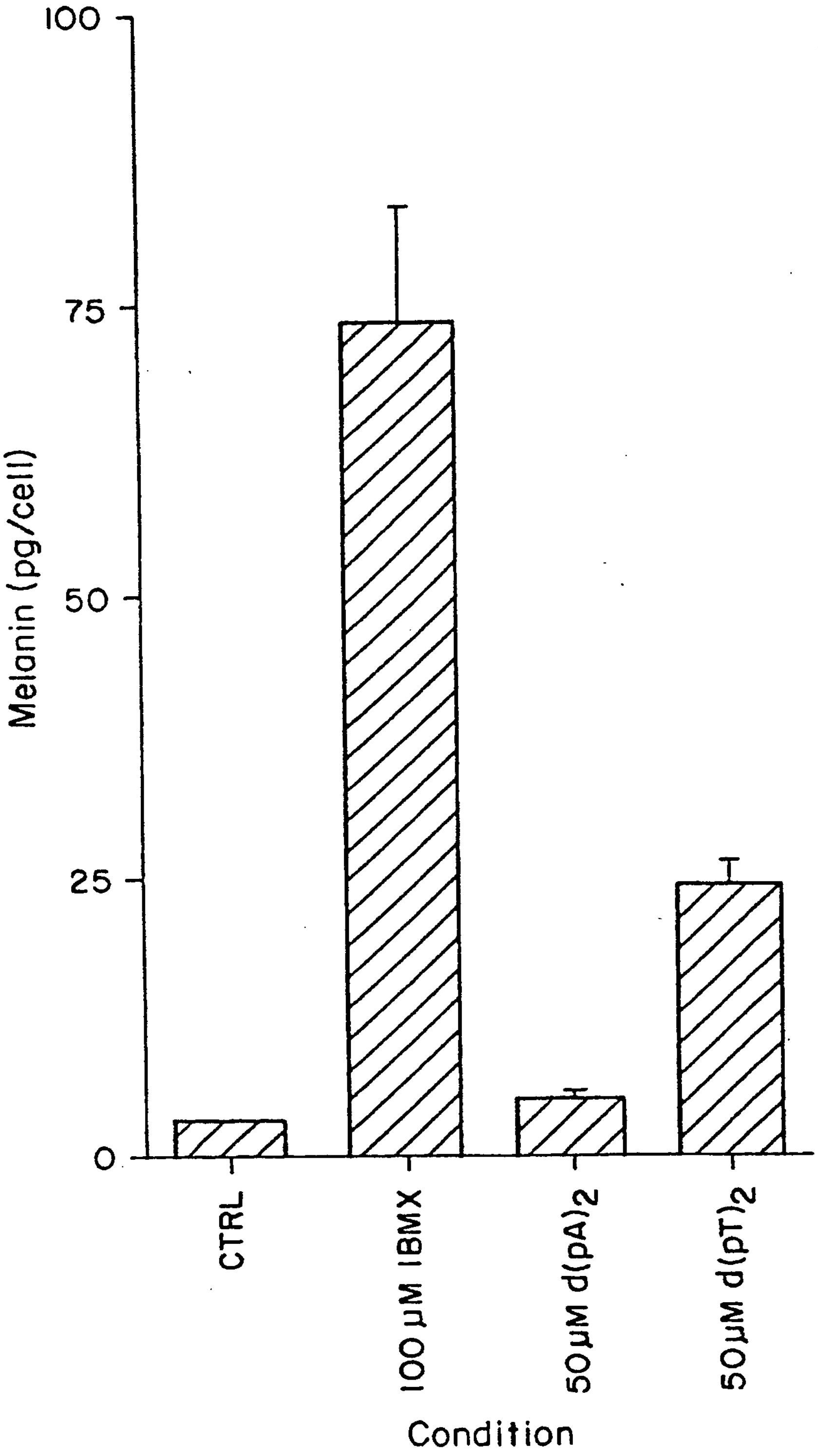
100
75
50
25
0
Melanin (pg/cell)
CTRL
100 μM IBMX
50μM d(pA)2
50μM d(pT)2
Condition

FIG. 3

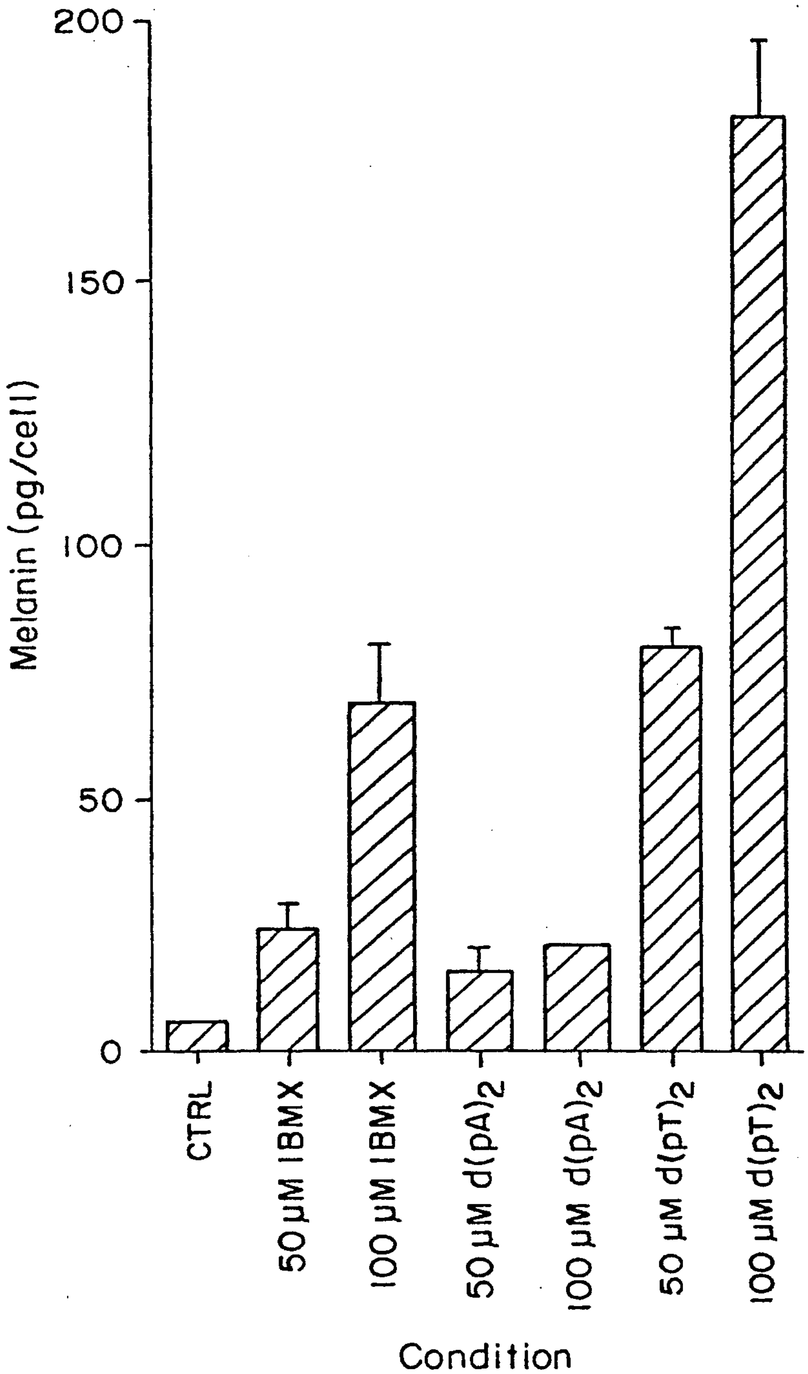
200
150
100
50
0
Melanin (pg/cell)
CTRL
50 µM IBMX
100 µM IBMX
50 µM d(pA)2
100 µM d(pA)2
50 µM d(pT)2
100 µM d(pT)2
Condition

FIG. 4

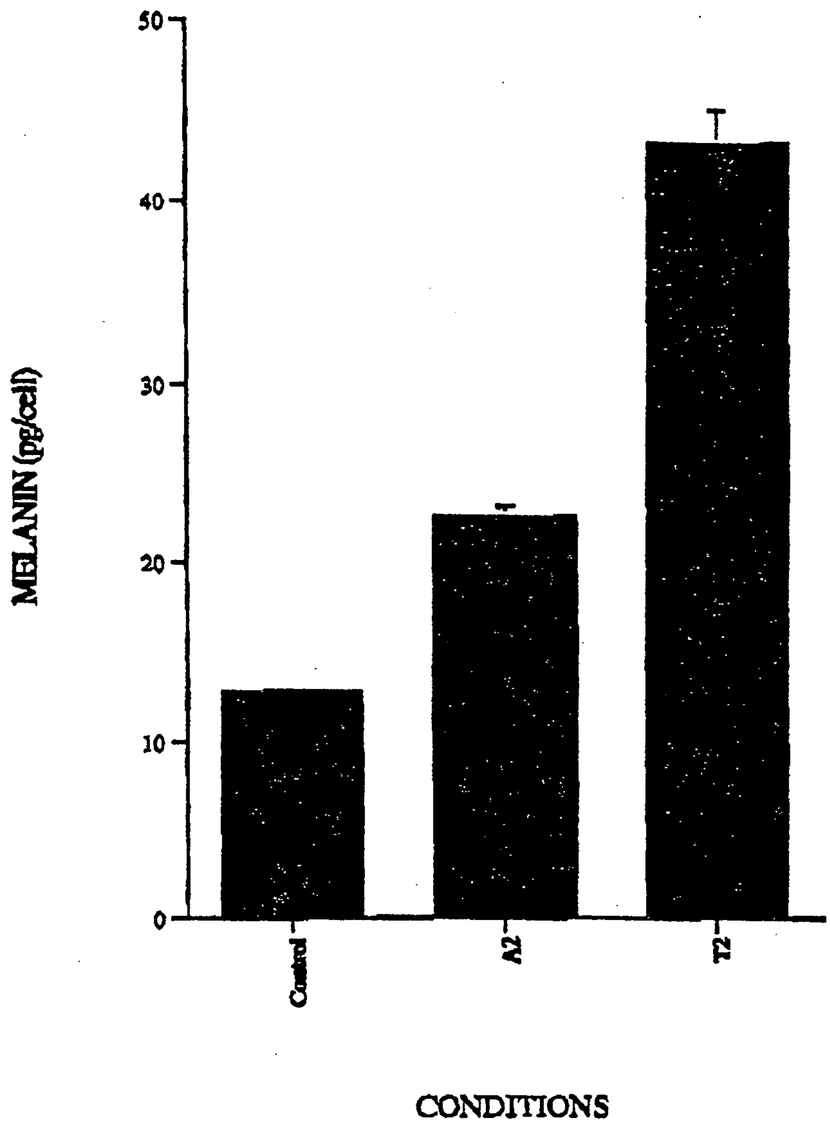

FIG. 5

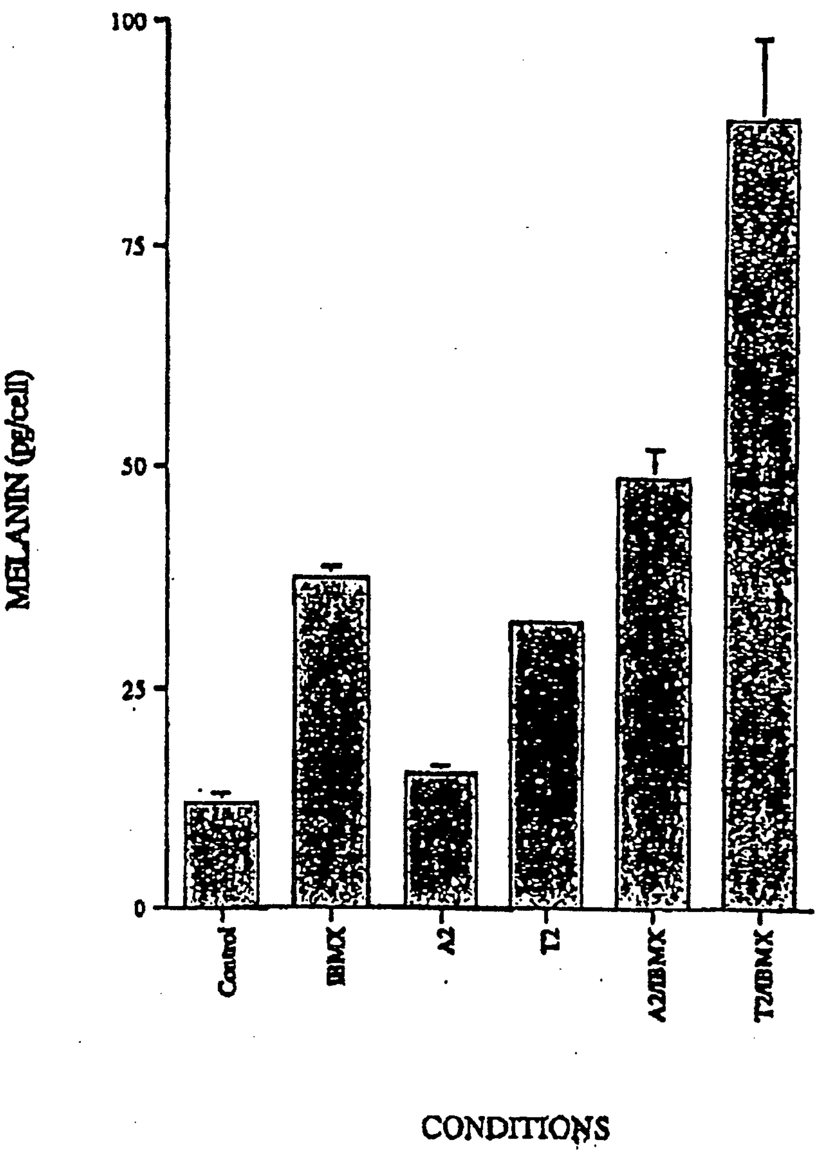
100
75
50
25
0
MELANIN (pg/cell)
Control
IBMX
A2
T2
A2/IBMX
T2/IBMX
CONDITIONS

FIG. 6

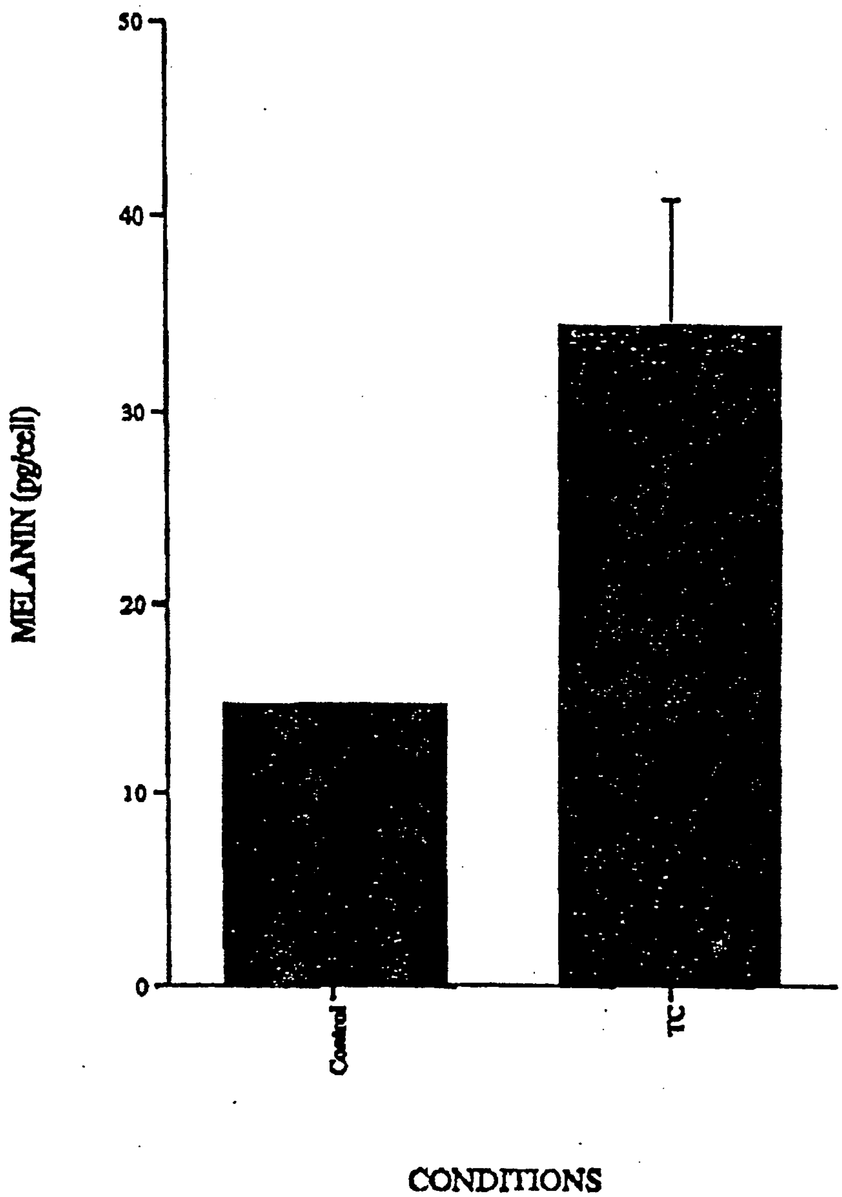

FIG. 7

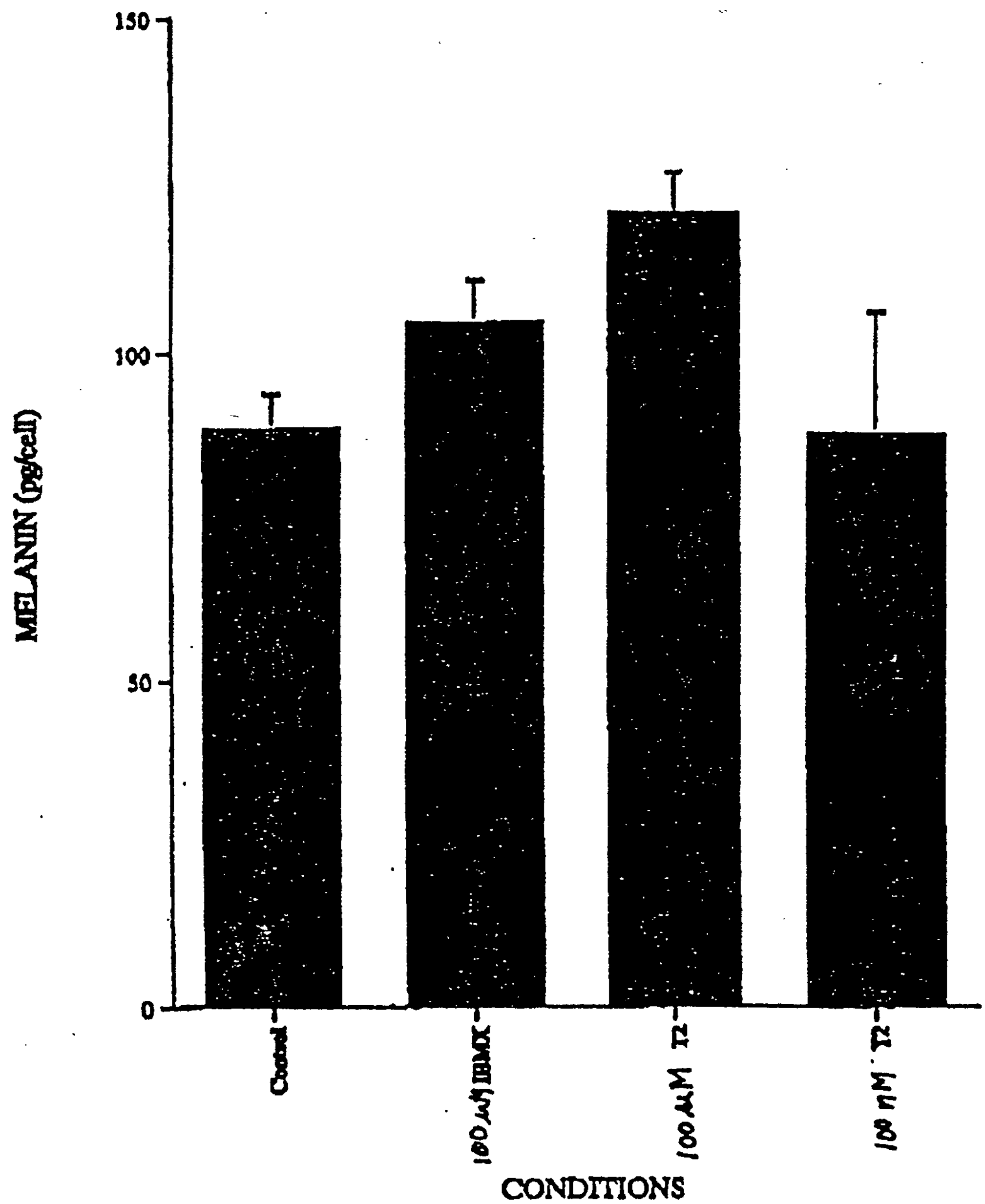
150
100
50
0
MELANIN (pg/cell)
Control
100 µM IBMX
100 µM T2
100 nM T2
CONDITIONS

FIG. 8

INTERNATIONAL SEARCH REPORT

Inter   nal Application No
PCT/US 94/07647

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K7/48 A61K7/42 A61K31/70 C12N5/00 //(A61K31/70, 31:52,31:23)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FR,A,2 511 243 (D. GESKIS) 18 February 1983 see the whole document ---	1,2,13, 17,18,30
X	EP,A,0 416 677 (CRINOS INDUSTRIA FARMACOBIOLOGICA S.P.A.) 13 March 1991 see claims ---	18,19, 25,30
Y	EP,A,0 484 199 (L'OREAL) 6 May 1992 see the whole document ---	1-33
Y	US,A,3 937 809 (O.K. JACOBI) 10 February 1976 see the whole document ---	1-33

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 October 1994	07. 11. 94

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Orviz Diaz, P

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 94/07647

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	PATENT ABSTRACTS OF JAPAN vol. 13, no. 137 (C-582) & JP,A,63 301 810 (K. SUGIYAMA) 8 December 1988 see abstract ---	1-33
Y	PATENT ABSTRACTS OF JAPAN vol. 12, no. 462 (C-549) & JP,A,63 183 518 (K. SUGIYAMA) 28 July 1988 see abstract ---	1-33
Y	ANAL. SCI., vol.4, 1988 pages 215 - 217 Y. SUGITANI 'Red shift in photoacustic ultraviolet absorption spectra of solid purine bases, nucleosides and nucleotides.' see the whole document ---	1-33
Y	J. INVEST. DERMATOL., vol.97, no.4, 1991 M. YAAR 'Human melanocyte growth and differentiation: A decade of new data.' see the whole document ---	1-33
Y	J. SOC. COSMET. CHEM., vol.41, 1990 pages 85 - 92 D.B. YAROSH 'Enhancement of DNA repair of UV damage in mouse and human skin by liposomes containing a DNA repair enzyme.' see the whole document -----	1-33